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Abstract

It is said blastformation can hardly be observed in the tissue culture of mouse lymphocytes. However, in our experiments of mouse lymphocytes (obtained either from axillary or cervical lymph nodes) mixed with various cells in combination of other cells as A+C3H, A+C57BL, or C3H+C57BL, it has been verified that these lymphocytes readily undergo blastformation in the presence of PHA (phytohemagglutinin M) as adjuvant. In the single tissue culture of these lymphocytes without PHA, the blastformation is observable in 6 per cent of the cells, while in the presence of PHA it is seen in 13. 7 per cent of the cells. In the cases of mixed cultures blastformation is observable in 14 per cent in the absence of PHA, whereas it is seen in 35.4 per cent in the presence of PHA. There is obviously a significant difference (p=O.OOI) in the blast. formation when cultured in the presence of PHA, and its reproducibility also proves to be quite high.

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IDENTIFICATION OF MOUSE H-2 ANTIGENS BY MIXED LYMPHOCYTE CULTURE IN THE PRESENCE OF PHA

I. BLASTFORMATION IN THE TISSUE CULTURE OF MOUSE LYMPH NODE CELLS IN THE PRESENCE OF PHA

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For attaining satisfactory results in the organ transplantation the important points to be borne in mind are not only the skill in transplantation and knowledge of appropriate immunosupressive agents but also the proper combination of tissues. This fact is amply testified by the past experiences in blood transfusion. Clinically, out of the histocompatibility testings, allogeneic lymphocytes in peripheral blood are so far extensively used for *in vitro* mixed lymphocyte culture testing. This is done on the basis of the finding that small lymphocytes, believed to be the final product after the termination of nucleic acid and protein syntheses, undergo blastformation and mitosis (1, 2) when these cells are cultured with other cells for a few days in the presence of various substances and antigens. However, this testing has not yet given any significant evidence to prove the difference in antigens between the mixed cells. To solve this problem, it would be necessary to study whether or not the blastformation in mixed cultures of various mouse lymphocytes of known histocompatibility antigens would reflect their histocompatibility difference. Here, however, arises a difficulty in that differing from peripheral lymphocytes, lymphocytes of animals like mouse, rat, guinea pigs and rabbits, have much lower immune activity even in the presence of phytohemagglutinin (PHA) (3). Contrary to general opinions, we have discovered that the addition of PHA-M as an adjuvant to the tissue culture of mouse lymphocytes potentiates the immune response, and enhances blastformation. This paper describes our findings on this point.

MATERIALS AND METHODS

Lymphocytes: The animals used were male adult mice (about 8 weeks old)

consisted of such strains as A strain (H-2^a), C3H (H-2^k), and C57BL (H-2^b) mice. From these animals cervical and axillary lymph nodes were removed after pealing off the capsules as carefully as possible not to injure blood vessels, cut into small pieces in Hanks solution to let lymphocytes released into the solution, and filtered through Tetron 80-mesh filter. Each filtrate was then washed with Hanks solution repeatedly three times by centrifugation each for 10 minutes at 2,000 rpm, and the cells so separated were suspended in the culture medium to be used as lymphocytes.

Culture medium: In preliminary experiment culture media of different compositions with various commerical materials were prepared and to each of these media lymphocytes were added to make the final count to be 100×10^4/ml or 50×10^4 cells/ml, and these were cultured in each medium at 37°C for 7—10 days, and unstained cell counts (4) and the rate of blast-like cell appearance were taken daily and noted the time and day of their appearance. As a result the most suitable medium for such experiments proved to be the one composed of TC-199: YLE: Hanks solution: BS (calf serum), 5:2:1:2 (v/v). Hence all the experiments were conducted with this medium.

PHA: Phytochemagglutinin used was the product of Difco Laboratories, Detroit, Michigan, U.S.A. One vial of this was dissolved in 5 ml medium and this PHA solution was added to the culture medium in the concentration of 1 per cent (v/v).

Antibiotics: Penicillin (TAKEDA Pharmaceutical K. K.). 200 units/ml, was added to the medium.

Culture method: Either in single or mixed culture 100×10^4 cells/ml of lymphocytes are added to the medium (in some mixed cultures the ratio is 50×10^4cells/ml: 50×10^4 cells/ml). Each group consists of 12 such test tubes, and is cultured stationary at 37°C for 72-hour interval, then 3 test tubes are taken from each group, these are shaken well with Vibro-shaker as to make a uniform cell suspension, 10-fold Hanks solution is added, centrifuged at 2,000—2,200 rpm, and then sedimented cells are taken on slide-glass to prepare specimens. After staining the cells with May-Giemsa stain the cells are classified into large ($>110\,\mu^2$), intermediate ($110\,\mu^2> >56\,\mu^2$) and small ($<56\,\mu^2$) cells (5).

RESULTS

1. *Single culture*

a) In the case without PHA the rate of intermediate and large cell appearance was 6 per cent.

b) In the presence of PHA the rate of intermediate and large cell appearance proved to be 13.7 per cent ($p=0.001$) (Table 1).

2. *Mixed cultures* Combinations were A+C3H, A+C57BL, or C3H+C57BL plus lymphocytes.

a) In the absence of PHA the rate of intermediate-large cell appea-

Table 1 Percentage of large and intermediate cells in unmixed cultures

Experiment No.	Strain	Percentage without phytohemagglutinin-M	Percentage with phytohemagglutinin-M
1	A	8.4	18.8
2	A	5.2	15.2
3	A	3.2	13.0
4	A	6.8	10.4
5	C3H	7.0	12.2
6	C3H	6.6	14.0
7	C3H	5.2	14.0
8	C3H	8.4	8.6
9	C57BL	6.4	19.2
10	C57BL	6.04	12.0
Average			13.74
			(P=0.001)

Table 2 Percentage of large and intermediate cells in mixed cultures

Experiment No.	Strain combination	Percentage without phytohemagglutinin-M	Percentage with phytohemagglutinin-M
1	A+C3H	17.2	50.2
2	A+C8H	9.2	26.6
3	A+C3H	15.8	24.2
4	A+C3H	12.6	30.0
5	C3H+C57BL	15.0	31.6
6	C3H+C57BL	16.2	46.2
7	C3H+C57BL	13.4	26.8
8	C3H+C57BL	12.0	39.2
9	C57BL+A	19.0	47.0
10	C57BL+A	10.2	32.4
Average		14.06	35.42
			(P=0.001)

rance was 14 per cent.

b) With addition of PHA the rate of intermediate-large cell appearance proved to be 35.4 per cent ($p = 0.001$) (Table 2).

3. The items 1 and 2 are each repeated ten times and there can be observed significant difference and the reproducibility is quite high.

SUMMARY AND DISCUSSION

Recently, it has become clear that various substances induce blastformation of lymphoid cells in tissue culture. HIRSCHHORN (6, 7) reported

that among them lecithin, phytohemagglutinin (PHA) extracted from *Phaseolus vulgaris*, one of the species of beans, when added to the culture of human peripheral blood lymphocytes, induces 90—95% of the cells to form large and intermediate lymphoid cells, and 1 to 15% of these blast-like cells undergo cell division, while in the group without PHA only 5—10% of the cells are large and intermediate lymphoid cells, and also mitotic picture can be seen only in 0—0.2% of them. Since then Streptolysin S (8), Staphylococcal exotoxins (9), antileukemic antibody (10), including PHA, have been found to be non-specific stimulants to leucocytes, and bacterial products, viral products, non-protein allergens, as well as the allogeneic cells and cell homogenates as reported in the present paper are known to be specific stimulants (10).

In the tissue culture of mouse lymphoid cells alone, the blastformation amounts to only 6% of the cells in culture, whereas in the presence of PHA, the percentage of the blastformation is increased about two-fold. The blastformation in the culture of mouse lymphoid cells is markedly enhanced with addition of PHA, though its extent is much less than in the case of human peripheral blood lymphocytes. FIKRIG and associates (12) stated that the tissue cultures of rabbit lymphoid cells and peripheral blood lymphocytes, known to be difficult to undergo blastformation, show about 40% of them undergo the blastformation in the presence of PHA.

In the mixed cultures of a pair each consisting of two cell groups whose H-2 antigens differ; namely, in the mixed cultures of A+C3H, A+C57BL, or C3H+C57BL lymphoid cells, the blastformation can be observed in a higher percentage even without PHA than in the single cultures of them. This seems to be due to the competitive action of the histocompatibility antigens on the other group lacking such antigens. On the addition of PHA the blastformation of lymphoid cells is enhanced over two-fold, but it is probable that the non-specific stimulation effect of PHA might be potentiating the specific stimulation effect of lymphoid cells. According to the postulate of TALMAGE and PEARLMAN (13), all antigens have two-phase actions, one of which is a non-specific stimulation action that induces and accelerates the proliferation of the lymphoid cells of the host, and the other is a specific stimulating action that synthesizes and gives rise to corresponding antibody. It is said that every immune reaction initially induces the proliferation of non-specifically immunized lymphoid cells by its adjuvant effect, and subsequently a specific cell proliferation is elicited.

It seems reasonable to assume that our *in vitro* experiments have confirmed the theory postulated by TALMAGE and PEARLMAN. It is clear

that in the presence of PHA, the blastformation in mixed culture is enhanced over two-fold, be the combination allogeneic lymphoid cell culture or single cell culture.

CONCLUSION

It is said blastformation can hardly be observed in the tissue culture of mouse lymphocytes. However, in our experiments of mouse lymphocytes (obtained either from axillary or cervical lymph nodes) mixed with various cells in combination of other cells as A+C3H, A+C57BL, or C3H+C57BL, it has been verified that these lymphocytes readily undergo blastformation in the presence of PHA (phytohemagglutinin M) as adjuvant. In the single tissue culture of these lymphocytes without PHA, the blastformation is observable in 6 per cent of the cells, while in the presence of PHA it is seen in 13.7 per cent of the cells. In the cases of mixed cultures blastformation is observable in 14 per cent in the absence of PHA, whereas it is seen in 35.4 per cent in the presence of PHA.

There is obviously a significant difference ($p=0.001$) in the blastformation when cultured in the presence of PHA, and its reproducibility also proves to be quite high.

REFERENCES

1. Bain, B., Vas, L., and Lowenstein, L.: *Blood* **23**. 108, 1964
2. Bach, F., and Hirschhorn, K.: *Science* **143**, 813, 1964
3. Marshall, W. H. and Roberts, K. B.: Tuberculin-induced mitosis in peripheral blood leucocytes, *Lancet* **1**, 773, 1963
4. Schrek, R.: A method for counting the viable cells in normal and malignant cell suspension. *Amer. J. Cancer* **28**, 389, 1936
5. Kuroyanagi, T.: Thymus and immunity. *Japan Clinical Med.* (Nihon Rinsho) **22**, 2359, 1964 (in Japanese)
6. Hirschhorn, K., Bach, F., Kolodny, K. L., Firschein, I. L., and Has, J.: Immune response and mitosis of human peripheral blood lymphocytes *in vitro*, *Science* **143**, 1185, 1963
7. Hirschhorn, K., Firshein, I. L. and Bach, F. H.: Immune response of human peripheral blood lymphocytes *in vitro* histocompatibility testing, *Nat. Acad.* Washington, p. 131
8. Hirschhorn, K. and Ripps, C. S.: Lymphocytes interaction *in vitro* in isoantigen and cell interaction. Wistar Inst. Press, Philadelphia p **8**, 57, 1965
9. Ling, N. R., and Husband, E. M.: Specific and nonspecific stimulation of peripheral lymphocytes. *Lancet* **1**, 363, 1964
10. Grasbeck, R., Nordman, C., and Chapelle, A.: Mitogenic action of antileucocyte immune serum on peripheral leucocyte *in vitro*. *Lancet* **2**, 385, 1963
11. Bach, F. H., and Hirschhorn, K.: Lymphocyte interactions: A potential histocompatibility test. *Science* **143**, 813, 1964
12. Fikric, S. *et al.*: Culture of leucocytes from rabbit blood and lymph. *Proc. Soc. Exp.*

Biol. and Med. **112**, 379, 1966

13. TALMAGE, D. W. and PEARLMAN, D. S.: The antibody response. *J. Theoret. Biol.* **5**, 21, 1963